

Modulation of Oxidant Stress In Vivo in Chronic Cigarette Smokers

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Background Free radical-induced oxidative damage is thought to be involved in the pathogenesis of diseases associated with cigarette smoking. We examined the production of 8-*epi*-prostaglandin (PG) $F_{2\alpha}$, a stable product of lipid peroxidation in vivo, and its modulation by aspirin and antioxidant vitamins in chronic cigarette smokers.

Methods and Results We performed the following studies: (1) a cross-sectional comparison of smokers and control subjects, (2) an examination of the dose-response relationship, (3) an exploration of the effect of smoking cessation (3 weeks) and nicotine patch supplementation, (4) the effect of aspirin consumption, and (5) the effects of 5 days' dosing with vitamin E (100 and 800 U), vitamin C (2 g), and their combination. 8-*epi*-PGF $_{2\alpha}$ excretion (in pmol/minol, mean \pm SEM) was 176.5 ± 30.6 in heavy smokers, 92.7 ± 4.8 ($P < .05$) in moderate smokers, and 54.1 ± 2.7 ($P < .005$) in nonsmokers. Urinary levels fell from 145.5 ± 24.9 to

114.6 ± 27.1 (week 2, $P < .05$) and 112.6 ± 24.9 (week 3, $P < .05$) on cessation of smoking. Aspirin treatment failed to suppress urinary levels of 8-*epi*-PGF $_{2\alpha}$ despite a significant reduction in urinary 11-dehydro-TxB $_2$ production and suppression of 8-*epi*-PGF $_{2\alpha}$ and TxB $_2$ in serum. Vitamin C (pre, 194.6 ± 40.9 ; post, 137.2 ± 34.1 ; $P < .05$) and a combination of vitamin C and E (pre, 171.0 ± 39.8 ; post, 133.5 ± 29.6 ; $P < .05$) suppressed urinary 8-*epi*-PGF $_{2\alpha}$, whereas vitamin E alone had no effect.

Conclusions Urinary 8-*epi*-PGF $_{2\alpha}$ may represent a noninvasive, quantitative index of oxidant stress in vivo. Elevated levels of 8-*epi*-PGF $_{2\alpha}$ in smokers may be modulated by quitting cigarettes and switching to nicotine patches or by antioxidant vitamin therapy. (*Circulation*. 1996;94:19-25.)

Key Words • smoking • prostaglandins • free radicals • isoprostanes • 8-*epi*-PGF $_{2\alpha}$

Cigarette smoking exacts a continuing toll on the public health. Deaths caused by cardiovascular disease attributed to cigarette smoking in the United States are estimated at more than 150 000 annually.^{1,2} Among the mechanisms hypothesized to contribute to smoking-induced vascular damage is oxidant injury.³⁻⁵ Highly reactive elements in cigarette smoke facilitate DNA adduct formation^{6,7} and may directly induce platelet activation and vascular dysfunction.^{8,9}

A detailed understanding of oxidant injury in vivo has been precluded by the lack of reliable indexes of this process that are biochemically stable and susceptible to accurate quantification in a noninvasive manner.^{10,11} Traditionally, the susceptibility of lipoproteins to oxidation ex vivo,¹² the detection of chemical adducts ex vivo,¹³ and reliance on nonspecific or intermediate indexes of the process, such as measurement of malondialdehyde¹⁴ or conjugated dienes,¹⁵ have been used in clinical studies.

Recently, attention has focused on families of free radical-catalyzed isomers of arachidonic acid, the isoprostanes (Fig 1), as stable products of lipid peroxidation that circulate in human plasma and are excreted in urine.^{16,17} Using an estimate of F $_2$ isoprostanes based on

a PGF $_{2\alpha}$ internal standard, Morrow and colleagues have reported increased levels in cigarette smokers.¹⁸ The present study confirms and extends these findings.

We have developed a method to measure specifically 8-*epi*-PGF $_{2\alpha}$, an abundant F $_2$ isoprostane with mitogenic¹⁹ and vasoconstrictor¹⁷ capability. Excretion is dose-dependently increased in apparently healthy chronic cigarette smokers and falls when they switch to nicotine patches. Although 8-*epi*-PGF $_{2\alpha}$ may be formed in either a free radical- or a COX-dependent manner,²⁰ the increment in smokers in vivo is suppressed by antioxidant vitamins but not by aspirin. This contrasts with the smoking-related increment in thromboxane metabolite formation, reflective of platelet activation, which is suppressed by aspirin.

Methods

Clinical Trial Design

The clinical studies were performed at the Center for Cardiovascular Science at the Mater Hospital, a teaching hospital of University College in Dublin, Ireland. Informed consent was obtained from all subjects. Both nonsmoking and smoking volunteers were apparently healthy, with normal physical examination, biochemical screen, and full blood count. They reflected the ethnic catchment area of the hospital; all were white. The smokers ranged in age from 20 to 47 years (median, 27 years). No smokers or control subjects were taking any medications, including vitamins.

Five studies were performed. The first, a cross-sectional investigation, involved the collection of spot free-flow urine samples between 9 and 11 AM in 24 chronic cigarette smokers (18 men) who had smoked at least 15 cigarettes per day for the preceding 2 weeks. Corresponding samples were collected in 24 age- and sex-matched control subjects. All subsequent studies were carried out in volunteers from this first study. Their daily intake of cigarettes ranged from 15 to 45 (median, 25), and they had 4 to 40 pack-years of smoking (median, 15 years). The

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Selected Abbreviations and Acronyms

COX = cyclooxygenase
GC/MS = gas chromatography/mass spectrometry
PG = prostaglandin
TX = thromboxane

frequency of cigarette smoking was assessed by diary records and interview. Urinary cotinine, a stable metabolite of nicotine, was measured in urine samples as an index of nicotine consumption in volunteers. During these studies they smoked a single brand of cigarettes containing 1.2 mg nicotine and 12 mg tar.

The second study was a formal assessment of the dose-response relationship between the number of cigarettes smoked and urinary 8-*epi*-PGF_{2α}. Chronic smokers were brought to steady state by smoking either 15 to 30 cigarettes (median, 23) per day (moderate smokers; n=5) or >30 cigarettes per day (heavy smokers; n=5; range of number of cigarettes, 31 to 45; median, 38) for at least 7 days before collection of three successive 24-hour urine collections for measurement of 8-*epi*-PGF_{2α}, cotinine, and creatinine. The coefficient of variation of urinary 8-*epi*-PGF_{2α} for triplicate 24-hour samples in the 10 volunteers ranged from 0.5% to 15% (median, 2%). Samples were collected in age- and sex-matched nonsmoking volunteers. In 8 nonsmoking volunteers, the coefficient of variation for four 12-hour urine samples over a 2-week period ranged from 3% to 19% (median, 10%).

The third study was designed to assess the effects of smoking cessation on urinary 8-*epi*-PGF_{2α} in the cigarette smokers. Six male chronic smokers (age range, 20 to 47 years; median, 32 years) who had smoked >30 cigarettes per day for at least 2 weeks (range, 2 weeks to 10 years) performed two 24-hour collections for 8-*epi*-PGF_{2α}, creatinine, and cotinine just before quitting. They were placed on nicotine patches (Nicotinell, 21 mg/d; Ciba Geigy Pharmaceuticals) on completion of the collection and were maintained on them while urine was collected for analyses on days 13, 14, 20, and 21 after quitting.

We have previously described the increment in urinary thromboxane metabolites observed in apparently healthy cigarette smokers.²¹ This is reflective of platelet activation.^{21,22}

Prompted by this observation and the knowledge that 8-*epi*-PGF_{2α}, unlike other F₂ isoprostanes, may be formed by COX,²⁰ we assessed the effects of aspirin on urinary 8-*epi*-PGF_{2α} and 11-dehydro-TxB₂ in chronic cigarette smokers. Groups of moderate (n=5) and heavy (n=4) smokers collected 24-hour urine samples for analysis of 11-dehydro-TxB₂ and 8-*epi*-PGF_{2α} before (two collections) and after (two collections) dosing with aspirin 75 mg/d for 10 days. TxB₂ and 8-*epi*-PGF_{2α} were measured in serum samples obtained at the initiation of these urine collections. Healthy, nonsmoking control subjects (n=6) were randomized to receive either aspirin 325 mg as a single oral dose or a matching placebo. Urine (12-hour collections) and serum (at initiation of the urine collections) were collected before dosing and commencing 12 hours after drug administration.

Finally, the effects of short-term therapy with vitamins E and C were assessed in the smokers. Initially, five moderate smokers collected 24-hour urine samples for 8-*epi*-PGF_{2α} 3 days before and 2 days after receiving vitamin E (Roche Pharmaceuticals; 100 U/d for 5 days). Subsequently, a group of heavy smokers were allocated to receive (1) vitamin E 400 U twice per day (n=7), (2) vitamin C (Roche Pharmaceuticals) 1 g twice per day (n=5), and (3) a combination of the two vitamins at these doses (n=4). Urine (24-hour sample) and serum were collected before (two collections) and at the end of (two collections) the 5-day dosing periods. A 2-week washout intervened between treatments.

Biochemical Analysis

Urinary 8-*epi*-PGF_{2α},^{20,23} and 11-dehydro-TxB₂,²⁴ and serum TxB₂²⁵ were measured by GC/MS as we have previously described. Values were expressed as picomoles per millimole of creatinine (urine) or picomoles per milliliter (serum). Urinary cotinine was measured by radioimmunoassay,²⁶ and levels were expressed as nanomoles per millimole of creatinine.

Statistical Analysis

Sample size calculations were based on the desire to detect a difference of at least 50% with α=.05 and a power (1-β)=.9.

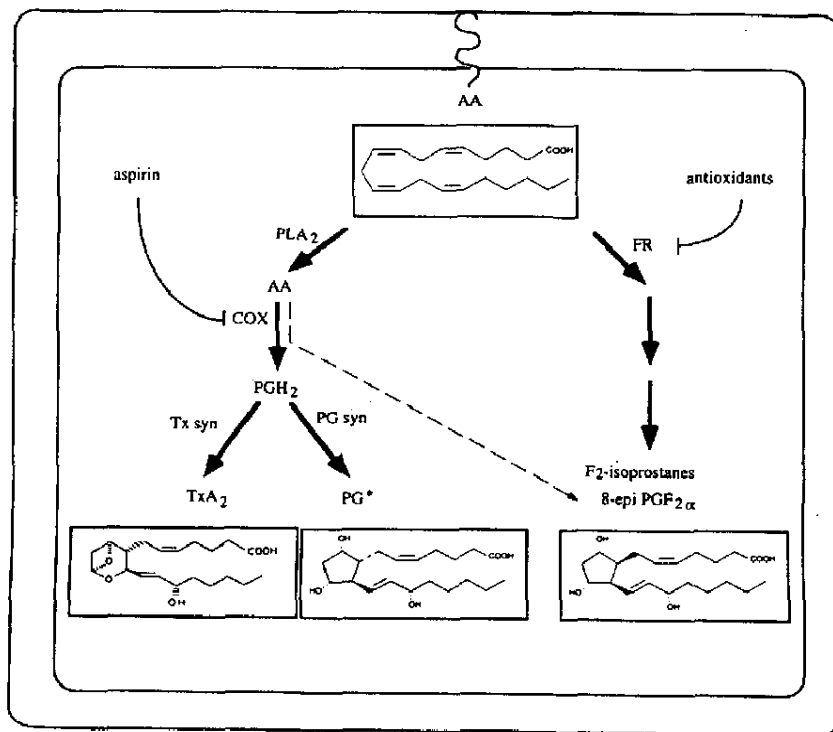


Fig 1. Membrane-bound arachidonic acid (AA) may undergo attack by free radicals (FR) in situ on phospholipids. Thermodynamic parameters control this chain reaction of peroxidation and reduction yielding 8-*epi*-PGF_{2α} and other stereoisomers of PGF_{2α} called isoprostanes (including PGF_{2α}), which, however, is not thermodynamically favored). Alternatively, AA, which has been cleaved from the membrane by phospholipase A₂, may be metabolized by a COX. Enzyme-dependent peroxidation and reduction yields PGH₂, which is subsequently metabolized to TxA₂ and other prostaglandins (*PGF_{2α} is illustrated here). 8-*epi*-PGF_{2α} is formed as a minor product of COX turnover in activated platelets. Aspirin prevents the COX-dependent formation of 8-*epi*-PGF_{2α}. Antioxidants might be expected to inhibit the free radical pathway.

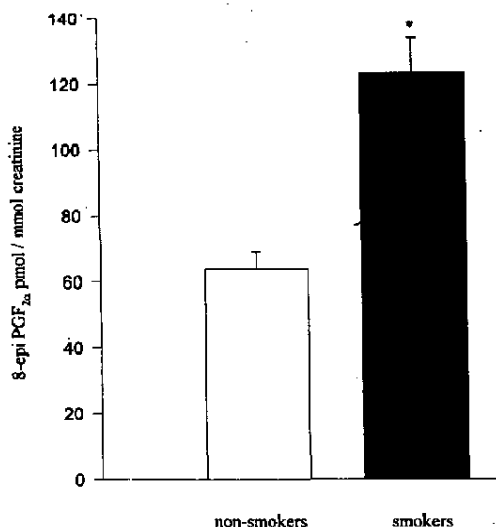


Fig 2. 8-*epi*-PGF_{2α} levels in spot urine samples of smokers (n=24) compared with age- and sex-matched control subjects (n=24; **P*<.005).

An unpaired *t* test was used when two samples were being compared. More than two samples were compared by a one-way ANOVA and, if significant differences occurred, by Duncan multiple range tests to assess where the difference lay. The within-cell median was used as a response variable for smoking cessation data and vitamin data and analyzed as a one-way ANOVA with repeated measures by use of a general linear model. All data are expressed as mean ± SEM, and differences were considered significant at a value of *P*<.05.

Results

Urinary 8-*epi*-PGF_{2α} excretion was significantly increased in the cross-sectional study (Fig 2) in the chronic smokers (122.5 ± 10.8 [mean ± SEM] pmol/mmol creatinine) compared with the age- and sex-matched nonsmoking control subjects (63.7 ± 5.0 pmol/mmol creatinine; *P*<.005).

A dose-response relationship was observed between the number of cigarettes smoked and both urinary cotinine and urinary 8-*epi*-PGF_{2α} (Fig 3). Heavy smokers had an average excretion of 8-*epi*-PGF_{2α} of 176.5 ± 30.6 pmol/mmol creatinine compared with 92.7 ± 4.8 pmol/mmol creatinine in moderate smokers (*P*<.05) and 54.1 ± 2.7 pmol/mmol creatinine in matched nonsmoking control subjects (*P*<.005). Smoking was a significant variable (*F*=28.4; *P*<.0001) in 8-*epi*-PGF_{2α} excretion. Furthermore, pairwise comparison indicated a significant difference between the moderate smokers and both the nonsmoking and heavy-smoking groups. Urinary cotinine levels were 2199 ± 376 nmol/mmol creatinine in heavy smokers compared with 630 ± 33 nmol/mmol creatinine in moderate smokers (*P*<.05) and 32 ± 3.2 nmol/mmol creatinine in nonsmokers (*P*<.005). The Pearson correlation coefficient for urinary values of 8-*epi*-PGF_{2α} and cotinine in all smokers was .46 (*P*=.09) (Fig 4). Thus, a tendency for 8-*epi*-PGF_{2α} to correlate with urinary cotinine failed to attain conventional statistical significance.

Urinary 8-*epi*-PGF_{2α} fell significantly on cessation of smoking in the heavy smokers (Fig 5A). Although the data exhibited heterogeneity, the levels fell from a precessation mean of 145.5 ± 24.9 pmol/mmol creatinine to 114.6 ± 27.1 (*P*<.05) at 2 weeks and remained sup-

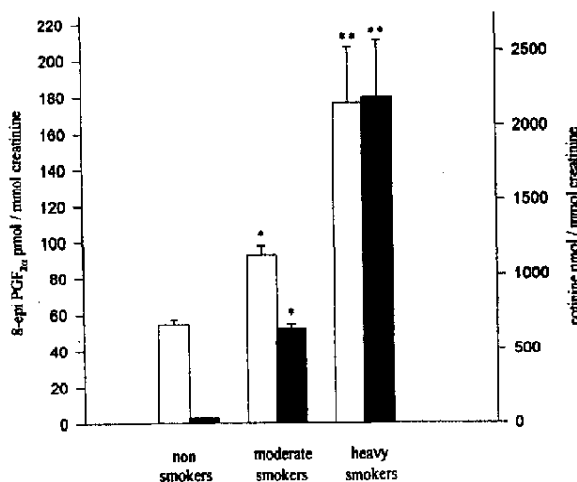


Fig 3. Higher levels of urinary 8-*epi*-PGF_{2α} (open columns) were seen in heavy smokers (n=5) compared with age- and sex-matched moderate smokers (n=5; **P*<.05) and with nonsmoking control subjects (n=14; ***P*<.005). Urinary cotinine levels (solid columns) were significantly higher in heavy smokers compared with moderate smokers (**P*<.05) and nonsmokers (**P*<.005).

pressed at 112.6 ± 24.9 (*P*<.05) 3 weeks after quitting. Smoking was a significant variable in urinary cotinine excretion (*F*=14.6; *P*<.0001); levels, as expected, fell after quitting (Fig 5B) and remained at a plateau level consistent with nicotine patch supplementation (precessation mean, 2312 ± 751 nmol/mmol creatinine; week 1, 982 ± 334; week 2, 928 ± 340; week 3, 871 ± 334).

Inhibition of COX by aspirin treatment significantly suppressed thromboxane biosynthesis in vivo, as reflected by urinary excretion of 11-dehydro-TxB₂, in all groups tested (Fig 6A). Levels (in pmol/mmol creatinine, mean ± SEM) fell from 158.4 ± 14.4 to 39.3 ± 10.4 in nonsmokers (*P*<.005), 200.8 ± 21.5 to 70.0 ± 9.2 in moderate smokers (*P*<.005), and 419.6 ± 21.8 to 105.9 ± 9.8 in heavy smokers (*P*<.005). Smoking was a significant vari-

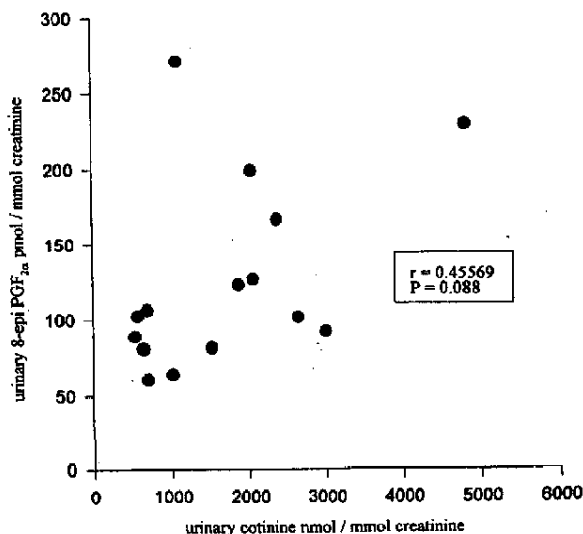


Fig 4. Correlation (*r*=.46; *P*=.09) of urinary 8-*epi*-PGF_{2α} and cotinine in chronic smokers (n=15). Each dot represents a different subject.

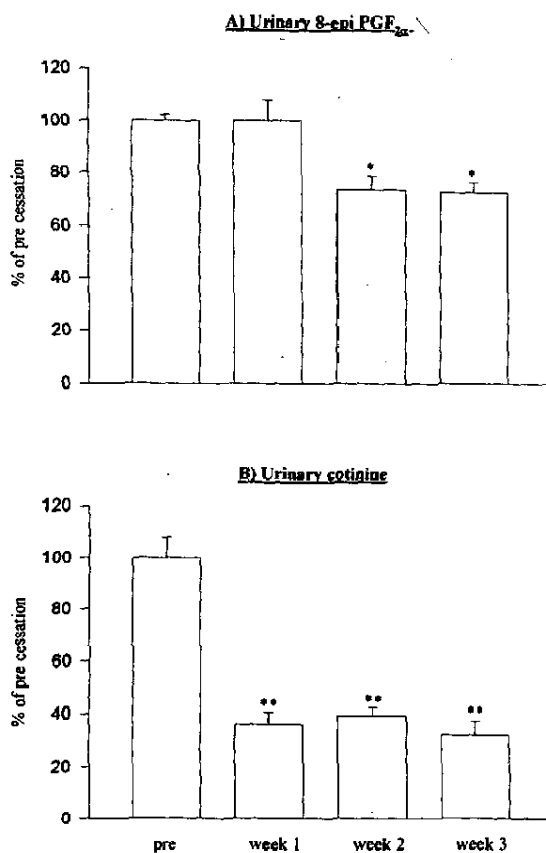


Fig 5. Percent change in the urinary levels of 8-epi-PGF_{2α} and cotinine in six chronic smokers after smoking cessation and nicotine patch supplementation. A, 8-epi-PGF_{2α} levels fell significantly by week 2 and remained suppressed through week 3 (**P*<.05). B, Cotinine levels dropped dramatically by week 1 (***P*<.005) and remained at a plateau through weeks 2 and 3.

able in urinary 11-dehydro-TxB₂ excretion before aspirin administration (*F*=46.3; *P*<.0001), and pairwise analysis revealed a significant difference between heavy smokers and both moderate smokers and nonsmokers. Concomitantly, aspirin reduced ex vivo serum TxB₂ production by >97% in all groups (Fig 7). Urinary 8-epi-PGF_{2α}, by contrast, was uninfluenced by aspirin administration even in heavy smokers who had significantly raised levels (*P*<.05) (Fig 6B). However, aspirin did suppress serum 8-epi-PGF_{2α} by roughly 80% ex vivo in moderate and heavy smokers (Fig 7). Urinary cotinine levels were similar before and after aspirin administration.

Vitamin E (100 U [moderate smokers] or 800 U [heavy smokers] per day) failed to suppress urinary 8-epi-PGF_{2α} excretion (Table). Vitamin C, by contrast, administered at 2 g/d, significantly depressed urinary 8-epi-PGF_{2α} alone and in combination with vitamin E (800 U/d).

Discussion

Isoprostanes belong to a family of free radical-catalyzed products of arachidonic acid.^{16,27,28} F₂ isoprostanes are formed in response to oxidant stress, initially in situ, via the formation of peroxy radicals of arachidonic acid in the phospholipid²⁹ (Fig 1). Subsequent cleavage and release into plasma appear to result from the action of a

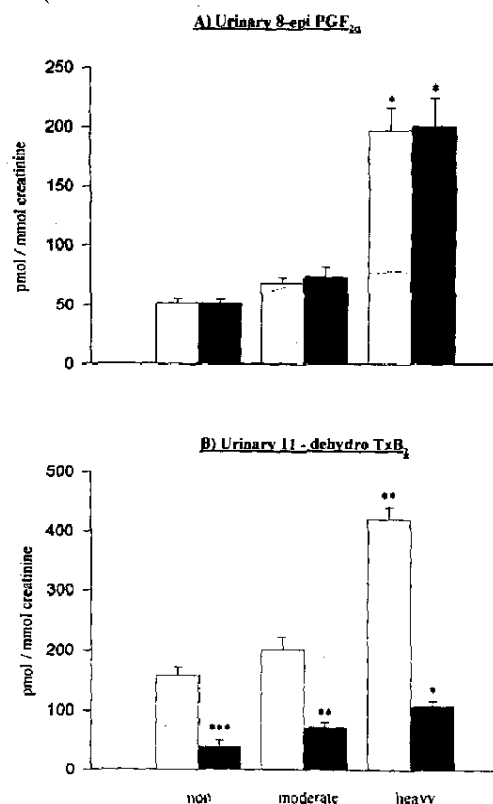


Fig 6. The effect of aspirin (pre, open columns; post, solid columns) in nonsmokers (*n*=6), moderate smokers (*n*=5), and heavy smokers (*n*=4) on urinary 8-epi-PGF_{2α} and 11-dehydro-TxB₂. A, Aspirin failed to suppress urinary 8-epi-PGF_{2α} excretion in any group. B, Urinary 11-dehydro-TxB₂ levels were significantly depressed by aspirin in all groups (***P*<.005).

phospholipase A₂. One of the more abundant F₂ isoprostanes, 8-epi-PGF_{2α}, exhibits biological activity. It is a vasoconstrictor in the renal and pulmonary vasculature and a mitogen in vascular smooth muscle cells.^{17,19,30} Although these effects are blocked by antagonists of the thromboxane receptor, 8-epi-PGF_{2α} differs from thromboxane analogues in its effects on platelets, in which it induces a shape change response but not irreversible aggregation.^{31,32}

Given its potential as both an endogenous ligand for the thromboxane receptor and a marker of oxidant stress in vivo, we developed a specific and sensitive assay for

Effect of Vitamin E, Vitamin C, and a Combination of the Two on Urinary 8-epi-PGF_{2α} Excretion

Treatment	Urinary 8-epi-PGF _{2α} , pmol/nmol creatinine		<i>P</i>
	Before Treatment	After Treatment	
Vitamin E, low dose	106.3±14.4	100.2±12.1	NS
Vitamin E, high dose	172.1±29.8	163.0±27.9	NS
Vitamin C	194.6±40.9	137.3±34.2	<.05
Vitamin E+C	171.0±39.8	133.4±29.6	<.05

Neither vitamin E in low dose (*n*=5 moderate smokers) nor in high dose (*n*=7 heavy smokers) reduced urinary 8-epi-PGF_{2α}, but both vitamin C (*n*=5 heavy smokers) and the combination of vitamins (*n*=4 heavy smokers) significantly suppressed urinary 8-epi-PGF_{2α} levels (*P*<.05). Values are mean±SEM.

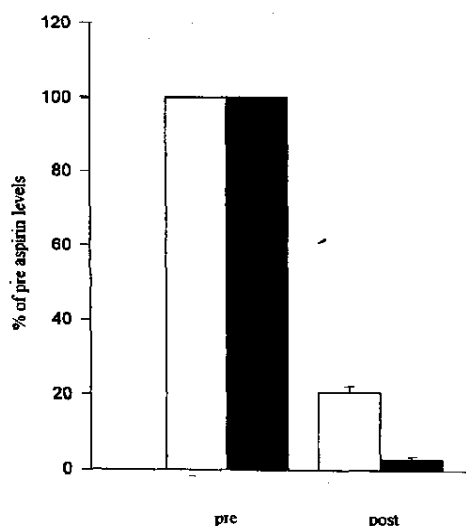


Fig 7. The percent change in serum 8-epi-PGF_{2α} (open columns) and TxB₂ (solid columns) in heavy smokers (n=4) treated with aspirin (75 mg/d for 10 days). Suppression of both compounds by aspirin was significant ($P < .01$).

8-epi-PGF_{2α}.²⁰ Previously, biosynthesis of F₂ isoprostanes has been estimated with an internal standard for the COX product of arachidonic acid, PGF_{2α}.¹⁶ Using this latter approach, Morrow and colleagues¹⁸ recently reported increased formation of these compounds in cigarette smokers. We performed a series of studies that extend these observations and support the utility of the specific measurement of 8-epi-PGF_{2α} as an index of free radical generation in vivo.

First, urinary 8-epi-PGF_{2α} is elevated in apparently healthy cigarette smokers compared with age- and sex-matched control subjects. Furthermore, this distinction was apparent whether the comparison was based on "spot" urine collections or on aliquots of varied duration (12 or 24 hours). Together with the availability of a GC/MS-validated immunoassay for this compound,³³ this observation greatly enhances the potential utility of this approach to the study of oxidant stress in vivo.

Second, there was a relationship between the number of cigarettes smoked and 8-epi-PGF_{2α} excretion. Thus, urinary 8-epi-PGF_{2α} was higher in individuals smoking more than 30 cigarettes per day than in those smoking 15 to 30 cigarettes per day. Urinary cotinine was also higher in the heavy smokers and tended to correlate with urinary 8-epi-PGF_{2α}. However, this particular relationship did not attain conventional statistical significance ($r = .46$; $P = .09$). This is unsurprising, because the effects of nicotine, of which cotinine is a urinary metabolite,³⁴ on 8-epi-PGF_{2α} may be quite distinct from those of cigarette smoking. Indeed, the precise constituents of cigarette smoke that contribute to 8-epi-PGF_{2α} excretion in smokers are unclear. Cigarette tar contains stable semiquinone free radicals, which, in aqueous solutions such as pulmonary fluid and plasma, are capable of reducing oxygen to superoxide, with subsequent dismutation to hydrogen peroxide.^{35,36} Metal ions in cigarette tar and body fluids can catalyze the formation of the hydroxyl radical from hydrogen peroxide via the Fenton reaction. Furthermore, the gas phase of cigarette smoke contains highly unstable organic radicals that are main-

tained in smoke for prolonged periods (>10 minutes). Nitric oxide and isoprene play a central role in this self-sustaining process by producing alkoxy and peroxy radicals.^{36,37} These, in turn, may react with superoxide in aqueous solutions to form peroxynitrite.

Finally, the degree to which free radicals might generate isoprostanes directly or via activation of monocytes, macrophages, and platelets, which, in turn, themselves generate free radicals, remains to be established. Thus, although both urinary cotinine and 8-epi-PGF_{2α} fell when the heavy smokers were switched to nicotine patches, this is consistent with both indexes reflecting consequences of cigarette smoking, rather than necessarily implying a causal relationship between them. Interestingly, on smoking cessation, urinary 8-epi-PGF_{2α} failed to decline to levels observed in nonsmokers. This implies that abstinence from smoking for 3 weeks reduces but does not abolish the oxidant stress associated with chronic, heavy cigarette smoking. Alternatively, it is possible that the ex-smokers were exposed to significant passive smoking in their homes or social environment. Indeed, a number of smokers did come from smoking households. However, the effect of such environmental influences on baseline 8-epi-PGF_{2α} excretion was not assessed formally.

8-epi-PGF_{2α} differs from other F₂ isoprostanes in that biological activity has been ascribed to it, as mentioned previously. It is unclear whether it might act as an incidental ligand at the thromboxane receptor,³⁸ activate a related but distinct receptor,¹⁹ or exhibit no autacoidal activity in vivo. 8-epi-PGF_{2α} does not activate the recombinant PGF_{2α} receptor in in vitro expression systems (A. Ford-Hutchinson, PhD, personal communication, 1994). Intuitively, it would be surprising if a product of lipid oxidation might have its own receptor. However, we have observed recently that 8-epi-PGF_{2α}, as distinct from other F₂ isoprostanes, can be formed in small amounts by the COX-1 enzyme in activated human platelets²⁰ (Fig 1). Thus, it is important to determine the extent to which an increment in urinary 8-epi-PGF_{2α} in cigarette smokers reflects free radical-catalyzed or COX-dependent formation of the compound in vivo. This is particularly true in the case of cigarette smoking, in which we have previously described elevated excretion of thromboxane metabolites, reflective of both platelet activation and increased COX turnover.^{21,22}

Inhibition of platelet COX-1 by aspirin consumption^{39,40} completely suppressed serum TxB₂ in the smokers ex vivo. Serum concentrations of 8-epi-PGF_{2α}, which are considerably lower than those of TxB₂ (733 ± 220 pmol/L versus 513 ± 70 nmol/L), are also suppressed by aspirin consumption by the smokers, but to a lesser extent than was the case with TxB₂ (mean, 82% versus mean, 98%). Serum 8-epi-PGF_{2α} levels were similar in moderate and heavy smokers at baseline (data not shown). This is not surprising, since there is precedence for equivalent serum levels despite significant differences in urinary production of TxB₂ in nonsmokers, moderate smokers, and heavy smokers.²¹ Thus, it appears that both a COX-dependent and a COX-independent component of 8-epi-PGF_{2α} generation are present in human serum. However, measurement of 8-epi-PGF_{2α} in serum samples heated to 37°C for 1 hour²⁵ is probably reflective of the capacity for product formation in serum rather than actual biosynthesis. Thus, serum concentra-

tions of TxB_2 exceed estimated endogenous plasma concentrations by roughly 2000-fold.⁴¹

Measurement of urinary 11-dehydro- TxB_2 is a validated index of thromboxane synthesis *in vivo*.^{25,42} We confirmed our previous observations²¹ of a dose-related increase in excretion of 11-dehydro- TxB_2 in apparently healthy cigarette smokers in the present study. However, while aspirin administration suppressed urinary 11-dehydro- TxB_2 , excretion of 8-*epi*- $\text{PGF}_{2\alpha}$ was unaltered. These results suggest that although the COX-dependent pathway may be a significant contributor to serum 8-*epi*- $\text{PGF}_{2\alpha}$ production *in vivo*, it remains a trivial component of overall 8-*epi*- $\text{PGF}_{2\alpha}$ biosynthesis, as reflected by urinary production. This holds true even in the setting of moderate COX activation, such as occurs in heavy cigarette smokers. Whether this holds true in settings of more marked platelet or neutrophil activation remains to be addressed. Similarly, although studies of the disposition of 8-*epi*- $\text{PGF}_{2\alpha}$ are under way, it is probable that, like that of urinary prostaglandin metabolites, its utility will be as a noninvasive time-integrated index of total body biosynthesis of this isoprostane. The relative tissue contribution to urinary levels would be expected to vary as a function of the disease under study.⁴³

The availability of a quantitative index of oxidant stress *in vivo* would permit investigation of the dose-response relationship of antioxidant drugs *in vivo* and their rational evaluation in relevant models of disease. For example, vitamins E and C exhibit antioxidant properties at concentrations used *in vitro*,^{44,45} but little information is available as to the antioxidant properties *in vivo* of minimal daily allowances, of doses used in pharmacological supplementation studies, or of the wide range of intakes incidental to consumption of unrestricted diets. It is perhaps unsurprising that attempts to define benefit derived *in vivo* from these and other antioxidant strategies have been frustrating, whether they involved interventional studies^{46,47} or population-based approaches.⁴⁸⁻⁵⁰

We used the chronic cigarette smokers to evaluate the consequences of short-term administration of two antioxidant vitamins on urinary 8-*epi*- $\text{PGF}_{2\alpha}$. In contrast to the effects of aspirin, administration of vitamin C, 2 g/d for 5 days, resulted in a significant decline of 8-*epi*- $\text{PGF}_{2\alpha}$ by an average 29%. A similar significant depression of 8-*epi*- $\text{PGF}_{2\alpha}$ excretion (mean, 23%) was observed when the smokers were treated with vitamin E 800 U/d in combination with vitamin C. Interestingly, vitamin E alone, at either 100 U/d (in moderate smokers) or 800 U/d (in heavy smokers), failed to suppress 8-*epi*- $\text{PGF}_{2\alpha}$ excretion significantly. The apparent efficacy of vitamin C is not surprising, given the depleted levels of this vitamin that have been demonstrated in chronic smokers.⁵¹ Furthermore, vitamin C has been shown to be superior to vitamin E in protecting plasma lipids and LDL from oxidative stress.^{45,52} This appears to be particularly important in plasma exposed to the gas phase of cigarette smoke, since lipid peroxidation is initiated only after vitamin C has been consumed.⁴⁵ Despite the inability of the hydrophobic vitamin C to suppress oxidation in lipophilic membranes, it recycles vitamin E within the lipid membrane, prolonging its antioxidant effect.⁵³ Thus, the relative deficiency of vitamin C in cigarette smokers may limit the antioxidant efficacy of vitamin E administered alone. Indeed, an increased rate of HDL oxidation has been reported in cigarette smokers who received supplemental vitamin E compared with re-

duced HDL oxidation in smokers administered a combination of vitamin E and vitamin C.⁵⁴ These observations illustrate the potential utility of 8-*epi*- $\text{PGF}_{2\alpha}$ in antioxidant dose-finding and have prompted the initiation of a more detailed investigation of the interaction of dose and duration of therapy with antioxidant vitamins on 8-*epi*- $\text{PGF}_{2\alpha}$ excretion.

Our results are consistent with the observations of two other groups. Those investigators used a measurement of either F_2 isoprostanes or 8-oxo-7,8-dihydro-2-deoxyguanosine, the latter thought to reflect repair of DNA and its precursors after free radical attack.^{18,55} Collectively, these results suggest that coincident with but distinct from platelet activation, apparently healthy individuals who smoke cigarettes exhibit abnormal levels of oxidant stress *in vivo*. The present study is also consistent with our finding of elevated urinary 8-*epi*- $\text{PGF}_{2\alpha}$ in other conditions putatively associated with oxidant stress, including reperfusion injury, adult respiratory distress syndrome, and poisoning with acetaminophen and paraquat.⁵⁶ Elevated 8-*epi*- $\text{PGF}_{2\alpha}$ in chronic smokers may be reduced by quitting smoking and switching to nicotine patches or by antioxidant vitamin therapy.

Acknowledgments

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